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BIOPROFILE SAMPLING

(Taken from the "Operations Manual" for Trip Interview Program [TIP] in the State/Federal Cooperative Statistics Program by James R. Zweifel, Date of Revision May, 1988 and the "Bioprofile Sampling Manual" by Barbara J. Palko, Date of Revision January, 1990. Originally modified for this manual December, 1992 and revised October 1999).

Various species are included in the **bioprofile sampling program**. This program works along with the TIP size-frequency data collection to provide biological samples for analysis of age, reproduction, feeding, and genetics. Ideally, the biological samples will be obtained from fish already randomly chosen for TIP size-frequency measurements; however, there will be cases where non-random sampling is necessary to meet targets for certain size groups or species. While the TIP database provides for housing of this data, non-random samples must be indicated as 'QS' or quota samples in section IV. In addition, samples will sometimes be taken from recreational fisheries. These must also be indicated as such by the appropriate Fishing Mode designation in section I.

A list of species and their priority ranking will be provided by separate memo along with the biological samples required for each species or species group. These biological sample target numbers are not the same as the target numbers for TIP size-frequency measurements. In most cases, the priorities will coincide with the size-frequency priorities; however, conflicts may arise. In these cases, the samplers need to contact their supervisors for instructions. Length, weight, and in some cases sex must be taken for every fish providing a biological sample. The memos will indicate samples which require sex identification. Samples can include otoliths or other hard parts (dorsal spines, scales, vertebrae, etc.), gonads, and tissues (heart, muscle, eye lens, etc.).

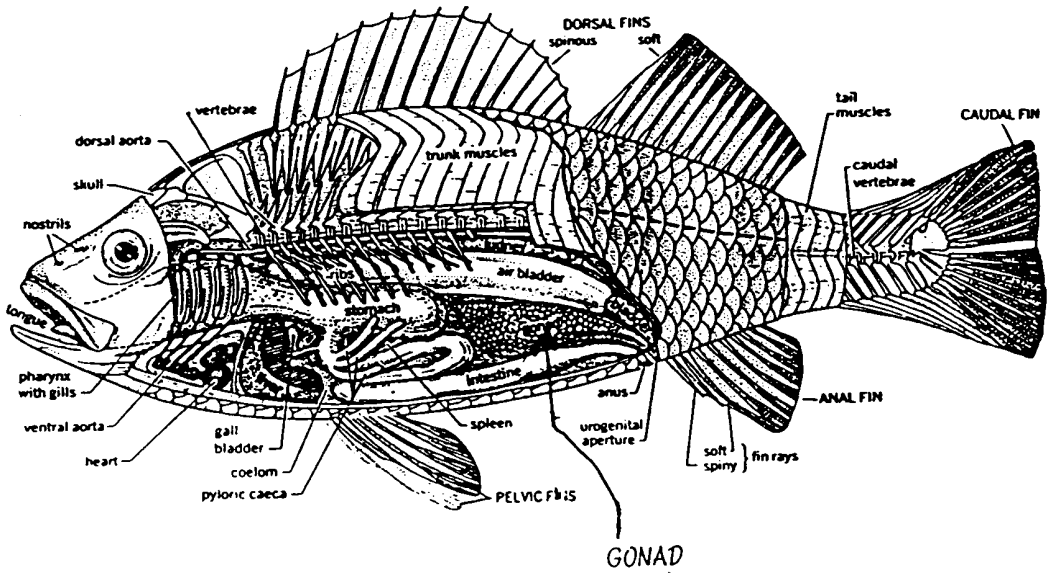

Figure 4.0

Figure 4.0 depicts a generalized drawing of a fish to provide a scheme to locate the various structures necessary to complete biological sampling. It is not meant to describe any specific species of fish.

LENGTH MEASUREMENTS

It is necessary to take length measurements on all fish which samples are taken from. These length measurements are taken during the course of TIP sampling and are recorded in the TIP database; however, **a summary of the lengths, sample tag numbers, and descriptions of samples taken must accompany the sample shipment to the respective lab.** These are provided on summary sheets. Summary sheets are provided (see Appendices). All lengths are to be fork (center-line) lengths and taken in millimeters.

Fish lengths can be measured on a board with a built in measuring scale or with a meter stick. The length of the fish is read from the board and either written on paper or recorded on tape. The punch board is a modification of the conventional measuring board and is more versatile in that it can be used to record lengths either by the conventional manner or by punching holes into the data sheet that overlays the board. The punch board system is described in Appendix H.

SEX DETERMINATION

Sex may be determined if samplers are allowed to open the body cavity or if the fish have been gutted and still retain a portion of the gonads. Sex should be recorded on the sample label using only one of three codes (M=male, F=female, U= unknown). This coding is for purposes of sample submission. Samplers may use a wider range of sex codes in TIP data submission (see Appendix A). Length and weight must be recorded along with the sex. Sex can usually be determined by macroscopic inspection of the viscera.

The ovaries are paired sausage shaped organs suspended from the dorsal wall. They are round to elliptical in cross-section and contain a central cavity or lumen into which ripe ova are shed. The color of ovaries vary from clear to whitish, to yellow-orange in ripening and ripe adults. As the ovaries become highly vascularized (many blood vessels) to accommodate increased blood flow during reproductive season, very ripe or spent ovaries take on a reddish color. Ovarian texture varies from smooth to slightly granular in young fish to grossly granular in ripe fish.

Like the ovaries, the testes are suspended from the dorsal wall within the body cavity. The testes are elongate, but are more elliptical to almost triangular in cross section and are without a lumen characteristic of ovaries. The testes vary in color from clear in the young to creamy-white in ripe adults: texture is smooth and the testes are frequently lobed in mature adults.

In less fresh specimens of both sexes, the color may fade or turn grey. When in doubt, cut a cross-section and note the presence/absence of the lumen characteristic of the ovaries in females. Lack of the lumen indicates testes/male. Both male and female gonads may be covered with large amounts of fat up to 100 X the gonad weight. This may cause ID problems. In these cases sex determination in the field will require close examination.

Correct sex determination is crucial in developing age-length keys for stock assessment, incorrect sex determinations can lead to erroneous conclusions regarding the status of a fisheries stock. If you are not sure of the sex , send a portion of the gonad on ice for verification, with the other biological samples for that fish. It can be difficult to macroscopically sex spent or undeveloped gonads.

For reef fish species, especially the groupers that change sex, gonads will have to be collected and shipped on ice for sex determination at a laboratory. There are often apparent differences among species. For example, triggerfish testes are quite small and atypical compared to other reef fish. Another reason to ship intact gonads, besides problems associated with staging, is for accurate weight and fecundity determination. Priorities for reproductive studies will also be indicated by separate memo.

POINTS IN SAMPLING GONADS

Open the body cavity by making a shallow cut from the anus to the throat area.

1. Care should be taken not to cut into the gonads. The use of rounded (gauze type) scissors with rounded end is a preventative against accidentally cutting into the gonads. Make insertion and cut from anus toward pelvic fins. After initial insertion of scissors, pull the skin away from the fish's underside as much as possible to reduce the chance of cutting a ripe ovary.

2. Immature gonads (from small fish) can be identified as a 2-lobed organ that is attached posteriorly in the abdominal cavity, dorsal to the anus.

3. Ziplock (heavy-duty, 2.7 mil, freezer type) bags should be used for storing and shipping the gonads. Remove excess air and effect an airtight seal before placing each gonad-bag between layers of ice. Each gonad should have a waterproof tag

including length, weight, date of landing and location. Gonads must not be held for more than 48 hours on ice. Gonads should be sent as soon as possible after collection and laboratory staff should be alerted if expected delivery will occur over a weekend or holiday.

4. Gonads should be shipped by Federal Express (overnight) in styrofoam boxes lined with 2 garbage bags and thoroughly layered in ice. The receiving Laboratory should be notified of all shipments and the number of boxes being shipped. **Please include copies of original data collection sheets.**

TISSUE SAMPLES

Tissue samples consist of 50 individual 1x2 inch pieces of tissue collected from 50 fish of a single species in a single day or within a maximum of 3 consecutive days, with their corresponding length, weight and sex information. Samples should be taken at set intervals which will be designated for each species. Muscle tissue samples can be collected by cutting a strip of flesh from the belly of the fish. Heart, liver, or eyes are removed accordingly. Each tissue sample should be put into a small pre-labeled plastic bag and iced down immediately. It is critical that all samples be frozen solid as soon as possible and kept frozen during shipment. Do not freeze gonad samples.

AGING STRUCTURE COLLECTION (otolith, head, dorsal spine)

Information and materials that must be recorded and used for aging structure sampling include all of the instructions stated for gonad samples. Aging structures (section V of TIP Reporting Form) include scales, otolith, spines, and/or vertebrae. Aging (structure) samples should be maintained separately from all other biological (gonad, tissue etc.) samples. Otolith or heads will be collected from king and Spanish mackerel, cobia and greater amberjack. For each species, port samplers will be told which structure (otolith, scale etc.) and quantity (quota)(number/size increment/gear type/location) to be taken. If otolith are taken instead of heads, they must be cleaned with fresh water and dried before being placed in vials for shipment. For the common dolphin, only dorsal spines (see Appendix H) will be sampled. Unless samplers are instructed otherwise, all scales will be taken beneath the tip of the posteriorly extended pectoral fin (Figure 4.1); at least 6-10 scales should be taken and stored in a scale envelope. Spines, when required, should be cut at the base (Figure 4.1) and stored in a scale envelope. Locations of otolith are shown in Figure 4.2. An otolith/head/spine sample is defined as one or more fish, one or more heads, or one or more pairs of otolith, or up to 6 dorsal spines from a single species taken from a single location by one gear type for a given date. A sample can include from one to twenty fish for each sex at 5 cm increments (for Spanish

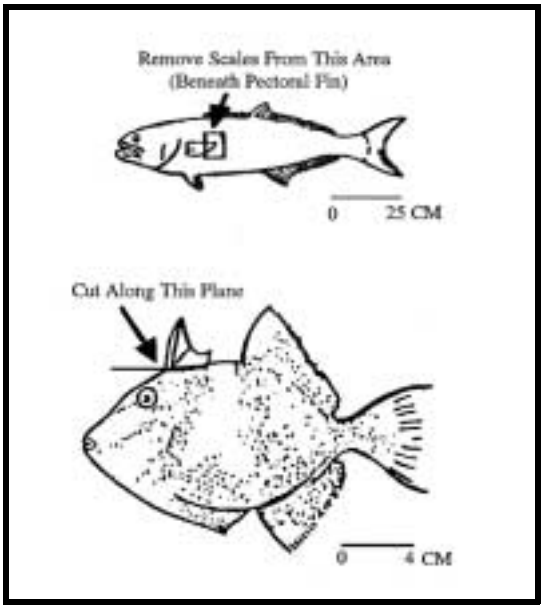

Figure 4.1

mackerel) or 10 cm increments (for all other species sampled).

If dorsal spines are to be sampled, collect the first 6 spines from the dorsal fin, cutting as close to the base as possible and from the leading edge of the dorsal fin (Figure 4-1). Place the spines in a pre-labeled small plastic bag and ice them immediately. Freeze them as soon as possible, and ship them by overnight express frozen. For our purposes, the otolith and spines will be used to determine the age of the fish in each fishery.

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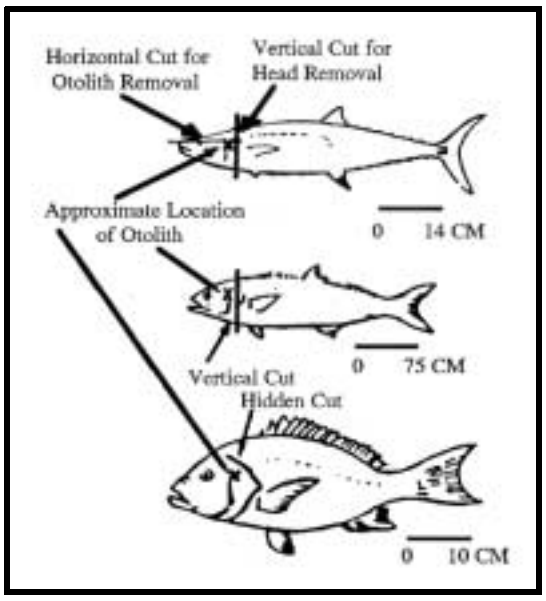

Figure 4.2

There are several ways to remove otoliths. The method will depend on the size of the fish and whether one needs to preserve the appearance of the fish. The following three methods have been used: (1) horizontal cut, (2) vertical cut, (3) hidden cut. See Appendix H for more detail on otolith location and removal.

HORIZONTAL CUT - This method is useful for large fish; otolith exposure is accomplished by making a horizontal incision with a sharp knife from the top of the eye posteriorly to the trunk (Figure 4-2). This can be done more efficiently by removing the head, and placing it on a solid object so that the snout is pointing upward, cut to expose the top of the cranial cavity. Use forceps to remove the two otoliths which are located posteriorly in the otic vesicles on both sides of the cranial cavity (Figure 4-2).

VERTICAL CUT - This incision allows otoliths to be removed from small fish by making a vertical cut behind the eye near the edge of the preopercle (Figure 4-2). The otic vesicles should appear as two small cavities on either side of the midline (Figure 4-2). If they are not visible, shave anteriorly until they appear. Use forceps to remove otoliths.

The vertical cut can be used when removing the head for later isolation of the otoliths. After making the vertical cut, remove any organs still attached to the head, and place head in bag and freeze. Use waterproof paper for labels.

When there are enough frozen heads to fill a styrofoam box, ship the box by U.S. Postal Express Mail, using the pre-paid express mailers. Line the box with 2 garbage bags (one inside the other) to prevent leakage and place all frozen heads inside these bags. **Make sure the heads are frozen.**

HIDDEN CUT - When the appearance of the fish is importance (marketing), the hidden cut or incision should be used. The dorsal insertion of the gill arch is severed. The operculum is then

lifted and the tissue is scraped away from the otic vesicle (Figure 4-2). In most fish a slight "bump" will coincide with the distal (outside) surface of the otic capsule. Gently remove the thin layer of bone using a sharp chisel to shave it off a little at a time. When the capsule is open, a cavity will appear with a visible white otolith. Remove the otolith with forceps. All otoliths must be cleaned with fresh water and dried before being placed in vials, stamped coin envelopes, or plastic bags. Aging structures can be shipped in the same box with their corresponding gonads. If no other biological samples were taken, then they can be shipped in a cardboard box along with copies of completed data sheets.

WHOLE FISH

When whole fish are shipped, length and sex measurements are not required. These measurements will be taken upon arrival at the Laboratory. A sample tag must be included with the tag number as indicated for **LABEL** below.

PROCEDURES FOR PRESERVATION OF SAMPLES

At the end of each day, all samples in plastic bags must be taken out and wrapped in gauze and placed on ice. Permanent labels must be securely tied to the gauze bags. Labels must also be inserted in vials if otolith were collected. Before storing each sample, labels for the viscera and aging structures should be checked against the TAG NUMBERS. All age structures collected must have corresponding length, weight etc. information from which the structures were taken.

REPORTING PROCEDURES FOR BIOPROFILE DATA

Agents must enter required TIP data (lengths, weights) that correspond to the fish sampled for bioprofile data (sex, gonad collections, and aging structures) and transmit by e-mail or diskette to Josh Bennett (joshua.bennett@noaa.gov), Southeast Fisheries Science Center-Sustainable Fisheries Division, 75 Virginia Beach Drive, Miami, Florida 33149. Biological samples along with copies of data sheets should be sent to the following:

Gulf of Mexico Biological Samples:	NOAA Fisheries - Panama City Lab Fisheries Profiles Branch 3500 Delwood Beach Road Panama City, FL 32408-7499
Atlantic Ocean (SE U.S. Coast) Samples:	NOAA Fisheries - Beaufort Lab Resource Ecology Branch 101 Pivers Island Road Beaufort, NC 28516-9722

Agents should complete the TIP reporting form, if any form is completed. Biological samples must contain a label with the following explanatory information:

LABEL Otolith/head/dorsal spines/tissue/viscera samples

Use only waterproof labels and use only #1 pencils for writing labels.

Interview number _____ **(Chap 3, Sec I, TIP Reporting Form Procedures)**
Date(mm/dd/yyyy) _____
Agent Initials _____
Species code _____ **(NMFS 4 digit or NODC 10 digit code)**
Sample number _____ **(Chap 3, Sec IV TIP Reporting Form Procedures)**
Tag number _____ **(Chap 3, Sec V, TIP Reporting Form Procedures)**
Length _____
Weight _____
Sex _____

It is of paramount importance that the TAG NUMBER recorded on this label match the tag number entered in section V, of the TIP data entry program.

Interview number, species code, agent, length and tag number (tag number's should be unique within the same sample). Care must be taken in recording the above information, the tag numbers entered on diskettes must match the tag numbers written on labels included with biological samples. These tag numbers will be used to match biological samples with other TIP information mentioned above.

Those agents that do not have a computer or the TIP Data Entry Program should send both TIP data (lengths, weights etc.) (on data form, if desired) and biological samples (with labels) directly to Panama City, Florida. Personnel at the Panama City Laboratory will be responsible for entering these data using the TIP Data Entry Program and sending these diskettes to Research Management Division (RMD) Miami, Florida.

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